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COMMENTARY

Exosome: from internal vesicle of the multivesicular body to intercellular signaling device

Kristin Denzer, Monique J. Kleijmeer, Harry F. G. Heijnen, Willem Stoorvogel and Hans J. Geuze*

Department of Cell Biology, Institute of Biomembranes and Centre for Biomedical Genetics, University Medical Center Utrecht, The Netherlands

*Author for correspondence (e-mail: h.j.geuze@lab.azu.nl)

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SUMMARY

Exosomes are small membrane vesicles that are secreted by a multitude of cell types as a consequence of fusion of multivesicular late endosomes/lysosomes with the plasma membrane. Depending on their origin, exosomes can play roles in different physiological processes. Maturing reticulocytes externalize obsolete membrane proteins such as the transferrin receptor by means of exosomes, whereas activated platelets release exosomes whose function is not yet known. Exosomes are also secreted by cytotoxic T cells, and these might ensure specific and efficient targeting of cytolytic substances to target cells. Antigen presenting cells, such as B lymphocytes and dendritic cells, secrete MHC class-I- and class-II-carrying exosomes that stimulate T cell proliferation in vitro. In addition, dendritic-cell-derived exosomes, when used as a cell-free vaccine, can eradicate established murine tumors. Although the precise physiological target(s) and functions of exosomes remain largely to be resolved, follicular dendritic cells (accessory cells in the germinal centers of secondary lymphoid organs) have recently been shown to bind B-lymphocyte-derived exosomes at their cell surface, which supports the notion that exosomes play an immunoregulatory role. Finally, since exosomes are derived from multivesicular bodies, their molecular composition might provide clues to the mechanism of protein and lipid sorting in endosomes.

Key words: Exosome, Multivesicular body, Sorting, Antigen-presentation

INTRODUCTION

Eukaryotic cells secrete proteins from the biosynthetic pathway by constitutive exocytosis of secretory vesicles or by regulated release of secretory or storage granules upon appropriate stimulation. Results from several groups, however, have recently indicated that the endocytic pathway is involved in an alternative pathway of secretion. Cellular compartments displaying intraluminal membrane-bound vesicles, collectively named multivesicular bodies (MVBs; Sotelo and Porter, 1959), can also fuse with the plasma membrane in an exocytic manner. Pan and Johnstone described this pathway more than a decade ago in differentiating red blood cells (Pan and Johnstone, 1983), and only recently has its presence in a larger group of different cell types been established. It results in the release of certain membrane and cytosolic proteins into the extracellular milieu. During exocytosis, 60–80-nm vesicles, termed exosomes, are released from the lumen of MVBs (Pan et al., 1985; Harding et al., 1983; Vidal et al., 1989; Johnstone et al., 1987; Raposo et al., 1996; Raposo et al., 1997b).

MVBs are part of the pleiomorphic endosomal system, which consists of primary endocytic vesicles, early endosomes (EEs), late endosomes (LEs) and lysosomes (reviewed by Geuze, 1998; Mellman, 1996). Within the endocytic tract, EEs are the major entry site for endocytosed material, whereas LEs are thought to receive newly synthesized lysosomal hydrolases directly from the trans-Golgi network. Lysosomes are the next station in the endocytic tract and, together with LEs, constitute the main site of protein and lipid degradation. The position of different subcompartments along the endocytic route can be determined on the basis of morphological characteristics, kinetics of fluid-phase marker uptake and differential distribution of marker proteins (Geuze, 1998; Mellman, 1996). The recycling plasma membrane protein transferrin receptor (TfR) is frequently used to identify early sorting and recycling endosomes. In some cells LEs contain the mannose 6-phosphate receptors, which deliver newly synthesized soluble lysosomal hydrolases to the endocytic system. Finally, lysosome-associated membrane proteins (Lamps/CD63) and acidic hydrolases (β-hexosaminidase/cathepsin D) are often-used lysosomal markers. However, the distinction between LEs and lysosomes is not sharp. Here we consider MVBs to belong to the LE/lysosomal category.

In this Commentary, we first discuss selective protein and lipid incorporation into the internal vesicles of endosomes during their formation. Next, we provide an overview of

different cell types that secrete exosomes with or without a known trigger, and speculate on probable functions. We focus particularly on exosomes derived from antigen-presenting cells, their potential physiological targets and possible usefulness in immunotherapy.

FORMATION OF MVBs

Exosomes derive from the internal vesicles of MVBs (van Deurs et al., 1993; Gruenberg and Maxfield, 1995). Even though a model for the formation of MVBs was proposed thirty years ago (Hirsch et al., 1968), the molecular mechanism underlying this process has remained unresolved. The internal vesicles are probably formed by inward budding, which creates a membrane-enclosed compartment in which the lumen is topologically equivalent to the cytoplasm (see Fig. 1; Pan et al., 1985; Vidal et al., 1997). Membrane proteins and lipids are recruited selectively from the limiting membrane of MVBs to inwardly budding vesicles. Incorporated membrane proteins are often destined for lysosomal degradation. For example, growth factor receptors are rapidly endocytosed upon ligand binding, sorted into the luminal vesicles of MVBs and, ultimately, targeted to lysosomes and degraded. In the case of the epidermal growth factor receptor, this sorting process depends on receptor kinase activity, and annexin-I could be an essential substrate (Felder et al., 1990; Futter et al., 1993).

Lipids also seem to be sorted in MVBs. For example, exogenously administered N-lissamine rhodamine B sulfonyl-phosphatidylethanolamine (Vidal et al., 1997) and lysobisphosphatidic acid (LBPA or BMP [bis-(monoacylglyceryl) phosphate]; Wherrett and Huterer, 1972) are efficiently incorporated into the internal vesicles of MVBs (Kobayashi et al., 1998). LBPA-rich membranes are thought to regulate intracellular cholesterol transport, presumably by acting as a collection and distribution device (Kobayashi et al., 1999).

Recent evidence from yeast and mammalian systems indicates that phospholipid metabolism is required for the formation of internal vesicles. In the mammalian melanoma cell line MelJuso, inward budding from the endosomal membrane can be inhibited by the phosphatidylinositol 3-kinase (PI 3-kinase) inhibitor wortmannin. This drug causes a swelling of the endosomal system (Fernandez-Borja et al., 1999) and the induced large vacuoles contain few internal vesicles, which are linked to the limiting membrane by membranous connections. This suggests that wortmannin inhibits the invagination and/or pinching off of intraluminal vesicles, which indicates that PI 3-kinase has a role in MVB morphogenesis. These results are consistent with molecular evidence obtained in yeast: Emr and co-workers found that PI metabolism is important for MVB formation (Wurmser and Emr, 1998; Odorizzi et al., 1998). Here, the product of PI 3-kinase, phosphatidylinositol 3-phosphate, is degraded in the vacuolar lumen after sorting of the lipid into vesicles generated by invagination of the vacuole membrane. In yeast mutants that fail to metabolize phosphatidylinositol 3-phosphate, small (40-50 nm) uniformly sized vesicles accumulate within MVBs. MVBs seem to follow two distinct pathways: either they fuse with lysosomes (Gruenberg et al., 1989; Futter et al., 1996; Mullock et al., 1998), which results in degradation of their contents, or they fuse with the plasma membrane, which results in the exocytosis of the internal vesicles into the extracellular space. The possibility that these processes involve distinct categories of MVBs cannot be excluded, and the final destination of MVBs might well be determined by the interaction of different SNARE proteins (Luzio et al., 2000), as protein transport in eukaryotic cells requires the selective docking and fusion of transport intermediates with the appropriate target membrane (Rothman et al., 1994; Bock and Scheller, 1999). The available data on specific v- and t-SNAREs that are involved in LE/lysosome secretion are limited. Only for a related phenomenon, the exocytosis of mast cell granules, were candidates for proteins involved in fusion events with the plasma membrane, SNAP-23 (Guo et al., 1998) and Vamp7 (Hibi et al., 2000), suggested. The presence of Vamp7 on late endosomes/lysosomes has indeed be demonstrated at the ultrastructural level (Advani et al., 1999). In fibroblasts, the secretion of conventional lysosomes is Ca^{2+} dependent (Rodriguez et al., 1997) and regulated by Syntaxin VII (Martinez et al., 2000). In several cell types, including T cells, platelets, dendritic cells and mast cells (Raposo et al., 1997a), fusion of MVBs with the plasma membrane and exocytosis of the internal vesicles is regulated by specific stimuli.

FORMATION OF EXOSOMES BY DIFFERENT CELL TYPES

Exocytosis of MVBs, and the consequent release of 40–90-nm vesicles, has been described for various cell types, including reticulocytes (Johnstone et al., 1987; Vidal and Stahl, 1993; Rieu et al., 2000), B- and T-lymphocytes (Raposo et al., 1996; Escola et al., 1998; Peters et al., 1989), dendritic cells (Zitvogel et al., 1998; Kleijmeer et al., 1998), mast cells (Raposo et al., 1997a), platelets (Heijnen et al., 1999), macrophages (M. J. Kleijmeer and H. J. Geuze, unpublished results) and alveolar lung cells (H. J. Geuze, unpublished results). These cells are mainly of hematopoietic origin (reviewed by Raposo et al., 1997b). Before discussing exosomes derived from lymphocytes and dendritic cells in more detail, we first discuss exosomes that are secreted by reticulocytes and platelets, and speculate on their possible functions.

RETICULOCYTES CLEAR TRANSFERRIN RECEPTOR BY EXOSOME RELEASE

Reticulocytes are immature anucleated erythrocytes that contain ribosomes and remnants of subcellular compartments such as mitochondria, the ER, the Golgi and the endosomal system. During differentiation, these internal compartments are lost, and several plasma membrane proteins are cleared. For example, mammalian erythrocytes loose all their TfR during maturation (van Bockxmeer and Morgan, 1979). In most cells, endocytosed TfR is efficiently recycled to the plasma membrane, but in reticulocytes it is targeted to MVBs (Pan et al., 1985; Harding et al., 1983). There, it is sorted to the internal vesicles, which are subsequently released into the extracellular milieu (Pan and Johnstone, 1983; Johnstone et al., 1987; Harding et al., 1984). Thus, exosomes derived from

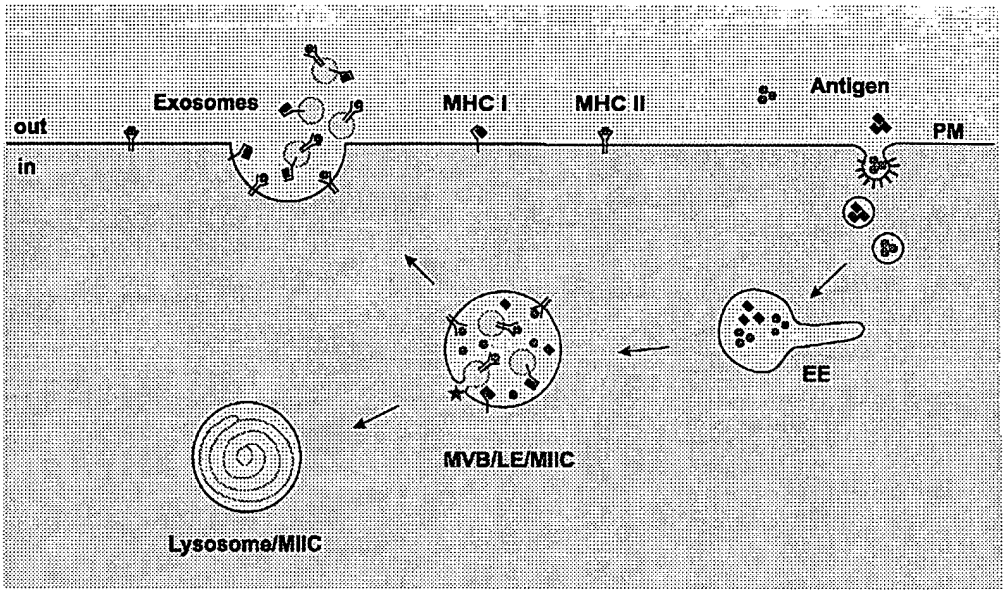

Fig. 1. Schematic representation of the formation of MVBs and exosomes in antigen presentation. Multivesicular bodies (MVBs) are late endosomal compartments (LE) situated in the endocytic route between early endosomes (EE) and lysosomes. Internal vesicles of MVBs are generated by inward budding of the limiting membrane (★). Exogenous antigens are internalized into the EEs of antigen-presenting cells (APCs) by receptor-mediated endocytosis (shown), phagocytosis or macropinocytosis. Acidification of the compartment activates proteases, which process antigen into peptides (black rectangles and blue dots). LEs and lysosomes of APCs harbor the majority of the intracellular MHC class II molecules and are therefore referred to as MHC-class-II-enriched compartments (MIICs). MHC class II molecules are found on the MIIC limiting (black) and internal (red) membranes, the latter being either vesicles (LEs) or sheets (lysosomes). MIICs also contain MHC class I molecules, which are predominantly located on internal vesicles. MIICs are a major site for peptide-loading of MHC class II molecules, and loading of recycling MHC class I molecules with exogenous antigen takes place in the endocytic tract. One way for the peptide-MHC to reach the plasma membrane is by fusion of multivesicular MIICs with the plasma membrane. In this way, MHC molecules are not only incorporated into the plasma membrane (PM) but also released into the extracellular space on the internal vesicles, which are then called exosomes. Exosomes contain peptide-loaded MHC molecules in an orientation that enables them to stimulate T lymphocytes in a haplotype- and peptide-restricted manner.

differentiating reticulocytes mediate clearance of obsolete proteins. Glycosylphosphatidylinositol (GPI)-anchored proteins, such as CD55 and CD59, are also efficiently sorted to exosomes (Rabesandratana et al., 1998).

Integrin α4β1, also known as VLA-4, is another protein that is downregulated during erythropoiesis and enriched on reticulocyte-derived exosomes (Rieu et al., 2000). The clearance function of exosomes is important in this case, because the presence of integrin α4β1 on reticulocytes can lead to blood circulation complications. Moreover, the presence of integrin α4β1 on the exosome surface allows exosomes to bind to endothelial cells through vascular cell adhesion molecule 1 (VCAM-1), which might represent another physiological function for the secreted vesicles.

PLATELETS SECRETE EXOSOMES UPON ACTIVATION

Blood platelets originate from megakaryocytes in the bone marrow and circulate in the blood in a non-adhesive state. Activation of platelets is triggered by surface adhesion (collagen) or specific agonists (thrombin) and leads to the release of the adhesive glycoproteins von-Willebrand factor and fibrinogen. Activated platelets secrete exosomes by fusion of alpha granules, which are the major storage site of the adhesive glycoproteins, and MVBs with the plasma membrane (Fig. 2; Heijnen et al., 1999). Additionally, they shed microvesicles from the plasma membrane, which are relatively large (>0.2 μm in diameter; Miyazaki et al., 1996) compared with exosomes (40-80 nm in diameter). Cell-surface-derived microvesicles and the plasma membrane of activated platelets have the same protein content and can both support coagulation (Fox, 1994). This procoagulant activity is provided by a negatively charged phospholipid surface (Zwaal et al., 1989), at which coagulation factors such as prothrombin and factor X assemble and become activated (Sims et al., 1989; Hoffman et al., 1992).

Stimulated platelets and isolated microvesicles have a high affinity for annexin-V, which indicates that the outer membrane leaflet is enriched in phosphatidylserine (Stuart et al., 1995). In contrast, platelet exosomes only poorly interact with annexin-V and do not bind prothrombin and factor X; therefore their extracellular function is probably not linked to coagulation (Heijnen et al., 1999). Platelet-derived exosomes are enriched in CD63, a tetraspan protein also found on exosomes from other cell types (see below). Tetraspan proteins have been implicated in adhesive as well as costimulatory and signaling functions. Platelet-derived exosomes are probably released at sites of vascular injury and could well function in the direct environment of adhering platelets. They are also present at the sites of contact between platelets and other neutrophils (H. Heijnen, unpublished observation), which suggests a role in heterotypic signaling.

EXOSOMES FROM CYTOTOXIC T LYMPHOCYTES

Cells of the hematopoietic system use lysosomes to store and release their secretory products (Stinchcombe and Griffiths,

1999). Cytolytic granules of cytotoxic $CD8^+$ T lymphocytes (CTL) are secretory lysosomes that have an MVB-like appearance (Peters et al., 1989). Immunoelectron microscopy has shown that the internal vesicles of CTL MVBs contain perforin and granzymes (Peters et al., 1991a). These proteins are the killing reagents, and their delivery needs to be tightly controlled. The internal vesicles also contain molecules such as the T-cell receptor (TCR), CD3 and CD8 that play roles in CTL–target-cell interaction. Recognition of target cells by the T cell receptor triggers kinesin-driven movement of the secretory lysosomes along microtubules to the point of membrane contact with the target (Griffiths and Argon, 1995). The membranes of the cytolytic granules then fuse with the plasma membrane, which results in the release not only of soluble proteins but also of small vesicles (Peters et al., 1989). The precise location and timing of release exocytic MVBs are tightly regulated so that they are perfectly focused on the target cell. This prevents damage of bystander cells, which are not recognized by the TCR. Peters et al., have postulated that the TCR-CD3 complex, CD8 and possibly other accessory proteins present in the internal membrane vesicles assist in the unidirectional delivery of the lytic substances to the target cell (Peters et al., 1990). Although the vesicles secreted by CTLs have not been referred to as exosomes previously, their formation and mode of exocytosis justifies such a classification.

EXOSOMES DERIVED FROM ANTIGEN-PRESENTING CELLS

Presentation of antigenic peptides by major histocompatibility complex (MHC) molecules to T cells usually takes place at the cell surface. However, antigen-presenting cells (APCs) have been shown to secrete exosomes that carry peptide-loaded MHC molecules, which can stimulate T cell proliferation in vitro (Raposo et al., 1996; Zitvogel et al., 1998). Two classes of MHC molecule present antigens to CTLs and $CD4^+$ T cells, respectively: MHC class I molecules and MHC class II molecules (Townsend and Bodmer, 1989; Germain and Margulies, 1993; Wolf and Ploegh, 1995). MHC class I molecules displayed on all nucleated cells present proteasome-processed peptides that are derived mainly from endogenous antigens to CTLs. By contrast, MHC-class-II-dependent antigen presentation is restricted mainly to APCs, such as dendritic cells, macrophages and B lymphocytes. These cells display exogenously derived peptides bound to MHC class II molecules to stimulate $CD4^+$ T cells. Exogenous antigens are internalized by either phagocytosis, (macro)pinocytosis or receptor-mediated endocytosis (Sallusto et al., 1995) and are proteolytically processed into 15–22-residue fragments in the endocytic pathway, where they bind to MHC class II molecules. The mechanisms involved in peptide-loading and trafficking of MHC class II molecules are reviewed elsewhere (Wubbolts and Neefjes, 1999; Villadangos et al., 1999; Nakagawa and Rudensky, 1999; Bakke and Nordeng, 1999).

The majority of intracellular MHC class II molecules reside in LEs and lysosomes, collectively called MHC-class-II-enriched compartments (MIICs; Fig. 3A,B; Peters et al., 1991b; Kleijmeer et al., 1996; Kleijmeer et al., 1997). MIICs are MVB-like or contain membrane sheets similar to those

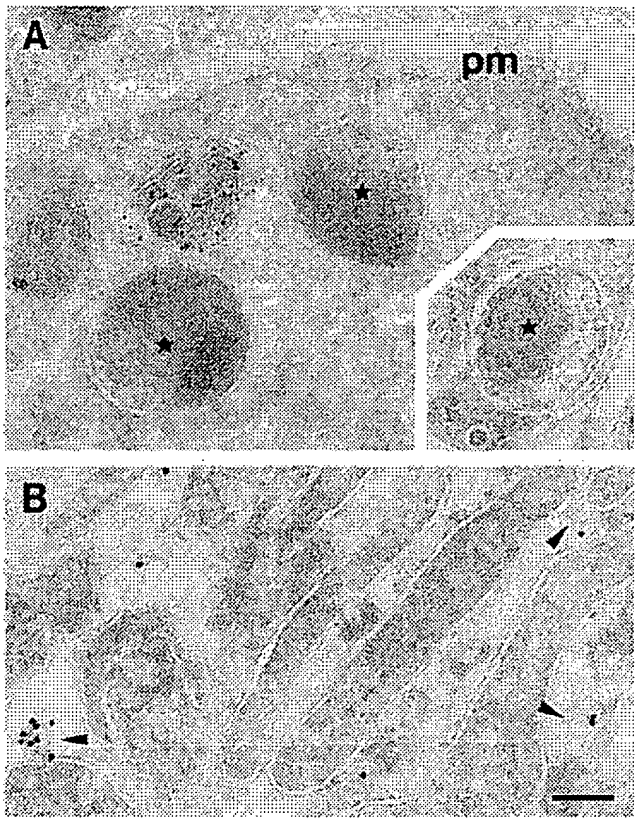

Fig. 2. Regulated secretion of exosomes in human platelets. (A) Immunoelectron micrograph of an ultrathin cryosection from a resting platelet. The figure shows two α-granules (★) and a multivesicular body. Label for the tetraspan protein CD63 (5 nm gold) is found on internal membrane vesicles. The inset shows small internal membrane vesicles situated at the eccentric rim of a secretory α-granule. (B) Immunoelectron micrograph of an ultrathin cryosection of a thrombin-stimulated platelet showing numerous pseudopodal extensions. CD63-positive exosomes (10-nm gold, arrowheads) are associated with the platelet cell surface (adapted with permission from Heijnen et al. (1999), Copyright The American Society of Hematology). Bar, 100 nm.

present in lysosomes of other cell types (Kleijmeer et al., 1995; Kleijmeer et al., 1997; Nijman et al., 1995) and contain the lysosomal marker proteins Lamp-1/2 and CD63, which are found predominantly in the multilaminar type. Experiments with human B lymphocytes allowed to endocytose BSA coupled to colloidal gold revealed that multivesicular MIICs are positioned earlier in the endocytic route than multilaminar MIICs (Kleijmeer et al., 1997). Using free-flow electrophoresis to fractionate mouse B-lymphoma cells and dendritic cells, Mellman and co-workers isolated an MHC class II peptide-loading compartment, the class-II-containing vesicle (CIIV), distinct from classical endosomes and lysosomes (Amigorena et al., 1995; Pierre et al., 1996). In a recent study, CIIVs have been proposed to represent organelles responsible for MHC class II transport from MIICs to the plasma membrane (Turley et al., 2000).

Internal and limiting membranes of MVB-type MIICs contain several distinct proteins. Tetraspan proteins such as

CD81, CD82 and CD63 are confined to the internal vesicles, whereas Lamps-1/-2 and HLA-DM reside primarily in the MIIC limiting membrane (Fig. 3A; Escola et al., 1998; Hammond et al., 1998). Sorting of MHC class II, CD63 and CD82 into MVBs, peptide loading onto MHC class II molecules and antigen presentation are defective in the lysosomal storage disease Chediak-Higashi-Syndrome (Faigle et al., 1998). Interestingly, the morphology of MVB-type MIICs is not affected, which indicates that formation of internal vesicles and loading with cargo proteins can be uncoupled. Tetraspanins are known to form large protein complexes and thus might facilitate incorporation of other proteins into the vesicles. Indeed, Vidal et al. have proposed that aggregation directs proteins into MVBs (Vidal et al., 1997).

MIICs also contain small amounts of MHC class I, predominantly located on the internal vesicles (Kleijmeer et al., 1998; Grommé et al., 1999). MHC class I molecules have recently been shown to have the potential to acquire exogenous antigenic peptides in the endocytic tract (Zitvogel et al., 1998; Grommé et al., 1999). This alternative peptide-loading pathway of MHC class I does not involve soluble proteins in general, but seems to be restricted to modified exogenous protein antigens – for example, those derived from virus-infected cells and tumor cells (reviewed by Jondal et al., 1996). Uptake of this antigenic material by APCs can involve engulfment of apoptotic bodies (Albert et al., 1998; Casciola-Rosen et al., 1996) or other membranous material, and results in the cross-priming MHC class I-restricted antigen-specific cytotoxic T lymphocytes. In this context, exosomes might function as intercellular vehicles for antigenic material.

DIRECT FUSION OF MIICS WITH THE PLASMA MEMBRANE RELEASES EXOSOMES WITH PEPTIDE-LOADED MHC MOLECULES

The classical traffic route from MIICs to the plasma membrane is thought to involve a vesicular transport step (Pond and Watts, 1997; Pond and Watts, 1999; Liu et al., 1998; Turley et al., 2000), but the vesicular intermediate has yet to be identified. We and others, however, have demonstrated that multivesicular MIICs, like MVBs in other cells, can fuse directly with the plasma membrane (Figs 1 and 3B) in B lymphocytes (Raposo et al., 1996) and melanoma cells (Wubbolts et al., 1996), which results in incorporation of MHC class II molecules into the plasma membrane (Raposo et al., 1996); another consequence is the release of exosomes displaying peptide-loaded MHC class II (see Figs 1 and 3C) and MHC class I molecules (Raposo et al., 1996; Escola et al., 1998; Zitvogel et al., 1998).

Table 1. Molecular composition of exosomes

	Functions	Reference for exosomal localization
TfR	Iron transport	Davis et al., 1986; Johnstone et al., 1987
Integrin α4β1	Adhesion/co-stimulation	Rieu et al., 2000
Mac-1/integrin α chain	Adhesion to target cells	Théry et al., 1999
MFG-E8/lactadherin	"	Théry et al., 1999
GPI-anchored proteins		
AchE		Johnstone et al., 1987
DAF/CD55	Regulation of complement activation	Rabesandratana et al., 1998
MIRL/CD59	Protection from complement mediated lysis/signaling	Rabesandratana et al., 1998
LFA-3/CD58 (GPI-isoform)	Adhesion/co-stimulation	Rabesandratana et al., 1998
Immunoglobulin-supergene family		
MHC class II molecule	Antigen presentation	Raposo et al., 1996, 1997a; Zitvogel et al., 1998
MHC class I molecule	"	Zitvogel et al., 1998; Kleijmeer et al., 1998
CD86/B7.2	Co-stimulation	Escola et al., 1998
CD54/ICAM-1	Adhesion to target cells	Théry et al., 1999
Tetraspan protein family		
CD9	Signal transduction/adhesion/complex formation with MHC and integrins	Théry et al., 1999
CD37	"	Escola et al., 1998
CD53	"	Escola et al., 1998
CD63/Lamp-3	"	Escola et al., 1998; Heijnen et al., 1999
CD81	"	Escola et al., 1998
CD82	"+HLA-DM/DO	Escola et al., 1998
Cytosolic proteins		
Hsp70	Protein folding	Mathew et al., 1995
Hsc73	Uncoating ATPase/peptide transfer to MHC	Théry et al., 1999
G_{i2} α subunit	Fusion	Théry et al., 1999
Annexin II	MVB formation	Théry et al., 1999
Lipids		
LBPA	Cholesterol transport	Denzer et al., 2000

These exosomes lack proteins normally present in the limiting membrane of MVBs, but are enriched in proteins that localize to internal vesicles of MVBs, such as MHC class I molecules, the costimulatory molecule CD86 and several tetraspan molecules (Nijman et al., 1995; Wubbolts et al., 1996; Escola et al., 1998, Heijnen et al., 1999; Hammond et al., 1998). The oligomer-forming tetraspan proteins have been implicated in antigen presentation, T cell signaling, T cell activation, cell motility and adhesion, and form complexes not only with one another (Angelisova et al., 1994; Berditchevski et al., 1997; Rubinstein et al., 1996) but also with HLA-DR (Angelisova et al., 1994; Hammond et al., 1998), MHC class I (Lagaudriere-Gesbert et al., 1997), integrins (Rubinstein et al., 1996; Fitter et al., 1999) and the T cell co-receptors CD4 and CD8 (Imai et al., 1995). Such large protein networks might limit the diffusion of molecules such as MHC class II and HLA-DM, thereby facilitating intermolecular interactions.

Analysis of dendritic-cell exosomes by mass spectrometry revealed that they contain several other proteins (Théry et al., 1999; see Table 1). A major component is the adhesion molecule milk fat globule EGF factor VIII (MFG-E8), which might target exosomes to integrins expressed by dendritic cells and macrophages. The cytosolic heat shock protein HSC73, a member of the HSP70 family, is another exosomal protein, which resides in the lumen of the exosome and is also present in dendritic-cell endocytic compartments (Théry et al., 1999). Exosomal heat shock proteins might play a role in transferring antigenic peptides, because, when isolated from tumor cells, they can induce specific antitumor responses (Udono and Srivastava, 1993).

EXOSOMES STIMULATE T LYMPHOCYTES IN VITRO

Exosomes derived from a human EBV-transformed B cell line (RN) exhibit abundant MHC class II molecules at their surface. The latter are functional, since exosomes loaded with specific antigens can activate $CD4^+$ T cell clones in vitro – albeit 10-20 times less efficiently than B lymphocytes (Raposo et al., 1996). Studies reporting the 'shedding' of MHC class II molecules by murine B cell lines and transfected T cell lines as part of supramolecular particles containing membrane lipids might therefore have to be reinterpreted (Emerson and Cone, 1979; Emerson and Cone, 1981; Arnold and Mannie, 1999). Such particles were proposed to be shed from the plasma membrane, but could represent exosomes. The presence of non-cell-associated MHC class II molecules in mouse serum (Callahan et al., 1976) is consistent with this idea.

Exosomes can stimulate CTLs in an MHC-class-I-restricted manner. Exosomes from dendritic cells pulsed with acid-eluted tumor peptides activate specific $CD8^+$ T cell clones in vitro (Zitvogel et al., 1998). Again the stimulation by exosomes was less efficient than with stimulation by intact cells displaying peptide-loaded MHC class I molecules. Nevertheless, the finding that exosomes do have antigen-presenting capacity for both MHC classes has major implications.

POSSIBLE FUNCTIONS OF EXOSOMES IN VIVO

To get more insight into the functions of exosomes, we must determine their physiological target(s). There are several potential ways for exosomes to interact with a target cell: binding to the cell surface, fusion with the plasma membrane or endocytic uptake. Recently, we discovered that exosomes are present in abundance on follicular dendritic cells (FDCs) in human tonsil tissue, a cell type that does not secrete exosomes itself (Denzer et al., 2000). FDCs are accessory cells of the immune system essential for affinity maturation and immunoglobulin isotype switching of B cell clones during the germinal center reaction (Liu et al., 1996; Tew et al., 1993; Tew et al., 1997; Lindhout et al., 1997), in which they present antigens to B and T lymphocytes (Gray et al., 1991). Light microscopy revealed that MHC class II molecules are present at the FDC surface. However, FDCs do not synthesize MHC class II molecules; instead they passively acquire peptide-loaded MHC class II molecules from donor cells (Gray et al., 1991). The mechanistic basis of this phenomenon is not clear, but was suggested to involve plasma membrane fragments shed from B lymphocytes trapped by FDCs.

Our study of human tonsil FDCs revealed numerous 40–70-nm membrane vesicles associated with the surface of FDCs (Denzer et al., 2000). Interestingly, these vesicles contain MHC class II molecules, whereas the FDC plasma membrane itself lacks MHC class II molecules (Fig. 3D). This indicates that the vesicles do not fuse with the cell surface but are somehow firmly attached to it. The protein compositions of the FDC plasma membrane and the attached vesicles are distinct: the vesicles are enriched in exosomal marker proteins, such as several tetraspan proteins, and also contain LBPA, a lipid marker for LEs.

In vitro binding experiments indicate that purified B-lymphocyte-derived exosomes specifically bind to FDCs but not to other cell types (Denzer et al., 2000; Fig. 3E). We therefore propose that FDCs are a physiological target for exosomes. Whereas antigen-immunglobulin complexes retained by FDCs are pivotal for selection for high-affinity B lymphocytes, exosomes docked on FDCs might select and recruit specific T helper cells. In this way, FDCs might sieve and facilitate the interaction between matching B and T cells,

Fig. 3. Protein sorting in MVBs, exocytosis of exosomes and their binding to FDCs. (A) Immunoelectron micrograph of an ultrathin cryosection of a human B cell line. The section was double-immunolabeled for the tetraspan protein CD82 (15 nm gold) and Lamp-1 (10-nm gold). Several types of MIICs are shown containing CD82 predominantly on the internal membranes, whereas Lamp-1 is primarily located at the limiting membranes (reproduced with permission from Hammond et al., Copyright 1998, The American Association of Immunologists). (B) Section as in A showing an exocytic profile with exosomes (E) that are heavily labeled for CD82 with 10-nm gold particles. (C) Immunoelectron micrograph of an ultrathin cryosection of a human dendritic cell double-immunolabeled for CD63 and MHC class II with 10-nm and 15-nm gold particles, respectively. Exosomes (E) are detached from the plasma membrane. D) Microvesicles labeled for MHC class II are attached to the FDC plasma membrane (PM), which itself is devoid of label. (E) Immunoelectron micrograph of isolated FDC–B-lymphocyte cluster from human tonsil. The clusters were incubated with biotinylated B-lymphocyte-derived exosomes before fixing and processing for IEM. The ultrathin cryosection shows a dendritic process FDC adjacent to a B lymphocyte (B). Several biotin-labeled exosomes are attached to the FDC cell surface (10-nm gold particles). Bars: 100 nm (A-C,E); 50 nm (D).

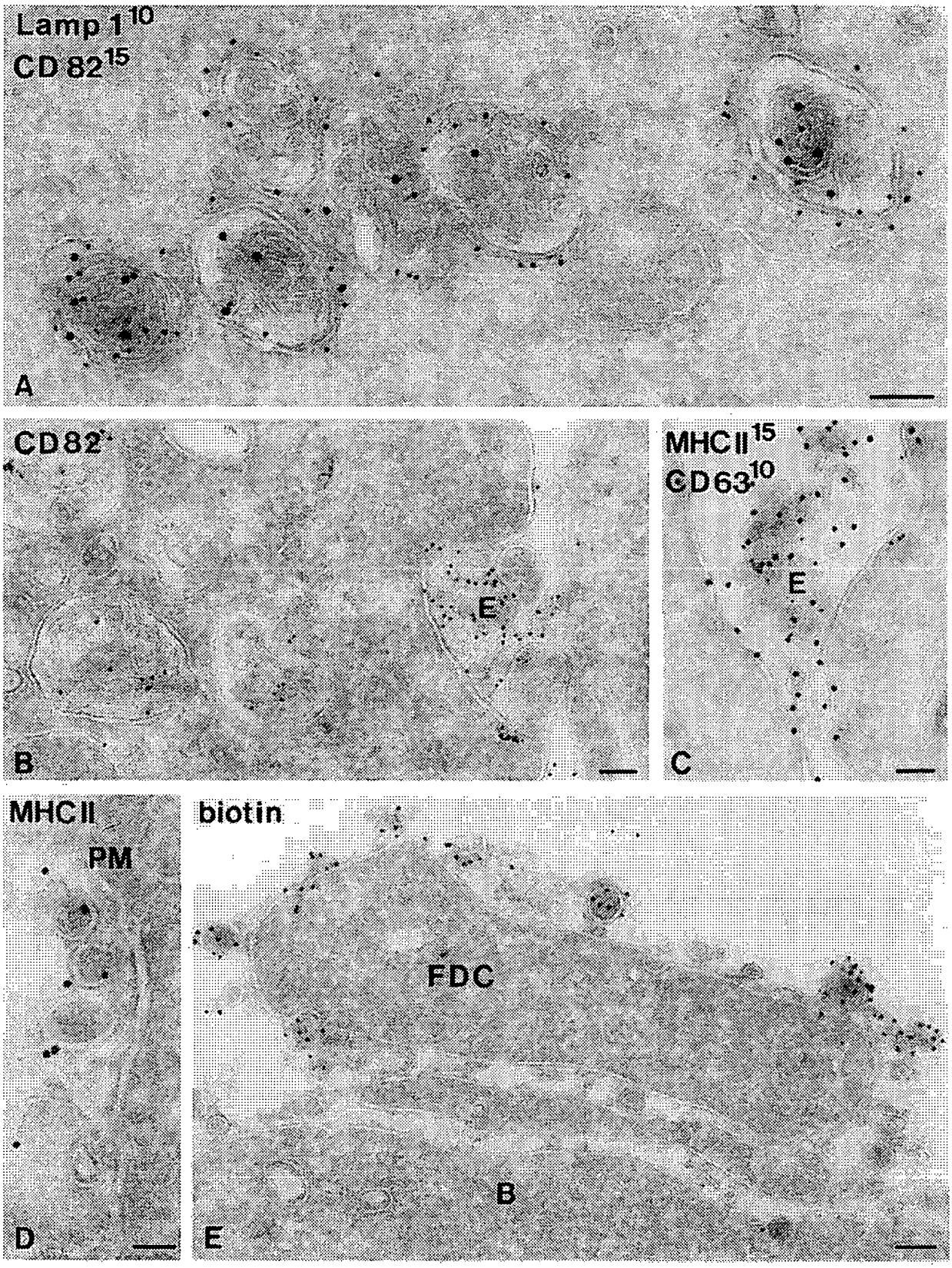
Lamp 1 10
CD 82 15
A
CD82
E
B
MHCII 15
CD63 10
E
C
MHCII
PM
D
biotin
FDC
B
E

Fig. 3

ultimately leading to isotype switching and their differentiation into plasma cells or memory B lymphocytes. In addition, exosomes might, depending on their half-life in vivo, serve an important role in long-term immune memory, a process that requires periodic antigen stimulation of memory T and B lymphocytes.

EXOSOMES AS IMMUNOTHERAPEUTICAL VEHICLES?

Even though their mechanism of function in vivo is not defined yet, exosomes might be an attractive immunotherapeutical tool combining the anti-tumor activity of dendritic cells with the advantages of a cell-free vehicle. Dendritic-cell infiltration of a primary tumor is associated with significantly prolonged survival and a reduced incidence of metastatic disease (Hart, 1997; Luykx-de Bakker et al., 1999; Timmerman and Levy, 1999). This finding has led to the development of a new strategy for cancer immunotherapy: dendritic-cell vaccination. Several studies have already shown that isolated dendritic cells loaded with tumor antigen ex vivo and administered as a cellular vaccine induce protective and therapeutic anti-tumor immunity in experimental animals (Shimizu et al., 1989; Flamand et al., 1994; Grabbe et al., 1991). In pilot clinical trials, dendritic-cell vaccination of patients with non-Hodgkin's lymphoma and melanoma induced anti-tumor immune responses and tumor regression (Luykx-de Bakker et al., 1999; Timmerman and Levy, 1999).

Zitvogel et al. have shown that not only do dendritic cells trigger T cell responses through direct cell-cell contacts, but exosomes secreted by dendritic cells can also stimulate T cells (Zitvogel et al., 1998). In this study, exosomes secreted by bone-marrow-derived dendritic cells, which were challenged with tumor-derived peptides, activated CTLs, causing the eradication of established tumors. The low efficiency of exosome-induced stimulation of T-lymphocytes in vitro compared with the dramatic effect of dendritic-cell-derived exosomes in vivo suggests that exosomes do not interact directly with CTLs. They may instead work as a transport vehicle for immunogenic antigens from immature dendritic cells located in the periphery to other dendritic cells, which get sensitized for T cell stimulation. Although, the mode of exosome action in vivo is not clear yet, exosomes produced by immature dendritic cells are a very interesting potential substitute for dendritic cells in tumor vaccination therapy.

CONCLUSION/PERSPECTIVES

During the past few years, it has become apparent that sorting events in multivesicular LEs result in the selective recruitment of proteins to the internal vesicles. These proteins are either destined to be degraded in the lysosome during differentiation of the cell or externalized in the form of exosomes for a variety of other functions. Studies on protein and lipid composition of exosomes are likely to reveal clues to the biogenesis of MVBs and sorting events in endosomes responsible for the specific composition of internal and limiting membranes. For the first time, such studies are within reach, because the internal MVB vesicles can quite easily be purified from the culture media of cells. Now that dendritic cell lines have become available, exosomes from these very potent primary APCs can be collected and probed in immunotherapeutical strategies. Up to now, FDCs are the only known physiological targets of exosomes; however, in vivo application of tagged exosomes from different cells in syngenic animals might reveal other targets.

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